Neutralization of Endogenous IL-1 Receptor Antagonist Exacerbates and Prolongs Inflammation in Rabbit Immune Colitis

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Abstract

Administration of exogenous interleukin-1 receptor antagonist (IL-1ra) is effective in reducing the severity of disease in animal models of acute inflammation. However, the function of endogenous IL-1ra in this process, is not yet known. We investigated the pathophysiological role of IL-1ra in a rabbit model of formalin–immune complex colitis. This model has previously been shown to be IL-1 mediated and a reduction in disease severity is observed with exogenous IL-1ra treatment. Colonic IL-1ra was found to be elevated subsequent to IL-1, and exceeded IL-1 levels 10-fold. Peak levels of IL-1ra preceded both the resolution of colitis and a significant decrease in IL-1 production. Administration of specific neutralizing antibodies against rabbit IL-1ra increased mortality and prolonged intestinal inflammatory responses. A significant increase in IL-1α colonic tissue levels was also measured as a result of exogenous anti–IL-1ra treatment. These studies are the first demonstration that endogenous IL-1ra may play an important role in regulating the host's inflammatory response by counteracting the deleterious and possibly lethal effects of IL-1 produced during acute inflammation. (*J. Clin. Invest.* 1994. 94:449–453.) Key words: interleukin-1 • acute inflammation • mortality • cytokine • inflammatory bowel disease

Introduction

Interleukin-1 receptor antagonist (IL-1ra) is a 22-kD cytokine that blocks the activity of IL-1 by competitively binding to IL-1 receptors and preventing IL-1–induced signal transduction in target cells (1–6). Two forms of IL-1ra have been described and are expressed in different cell types: a secreted form (sIL-1ra), primarily expressed in monocytes and macrophages and an intracellular form (icIL-1ra), which is associated with epithelial cells (7–10). The recent cloning and recombinant expression of sIL-1ra have allowed important advances in understanding the role of IL-1 in disease (11, 12). First, molecular probes have been developed and used to measure levels of endogenous IL-1ra in a variety of diseases. A comparison of IL-1 and IL-1ra levels has led to the hypothesis that the insufficient production of IL-1ra needed to counteract the observed increase in IL-1 is a potential mechanism in the pathogenesis of rheumatoid and Lyme arthritis, leukemia, psoriasis, and inflammatory bowel disease (IBD) (13–18). Second, recombinant human IL-1ra has been developed as a pharmacological agent to specifically inhibit endogenous IL-1 produced during such diseases. Administration of exogenous IL-1ra has been shown to be effective in reducing severity of inflammation in a variety of experimental models, presumably restoring the normal balance between IL-1 and IL-1ra, thereby supporting the putative role of IL-1 in the pathogenesis of these diseases (12, 19).

The precise role of endogenous IL-1ra during the host's response to acute inflammation and injury, however, remains to be elucidated. In particular, it is unclear whether endogenous IL-1ra has a role in regulating the biological activity of IL-1 produced in acute disease states. In the present study, we report that neutralization of endogenous rabbit IL-1ra exacerbates and prolongs inflammation in the rabbit model of formalin–immune complex colitis, suggesting that endogenous IL-1ra is an important mediator in downregulating acute inflammatory responses.

Methods

Rabbit colitis model and in vivo protocols. Inflammation was induced in the distal colon of male New Zealand rabbits (2–2.5 kg) by intrarectal administration of 4.0 ml 0.45% (vol/vol) unbuffered formaldehyde (Electron Microscopy Sciences, Fort Washington, PA), followed by i.v. injection of 0.85 ml immune complexes in antigen excess. The colitis model has been described in detail elsewhere (20–22). Animals were assigned to one of two protocols.

Protocol 1: time course study. Rabbits were sacrificed by lethal injection (40 mg/kg nembutal) (Abbott Laboratories, N. Chicago, IL) at 0, 4, 12, 24, 48, and 96 h, and 1 wk after induction of colitis. Longitudinal sections of distal colon were immediately frozen in liquid nitrogen and processed for histological assessment of inflammation and cytokine measurement as described previously (10, 22). Statistical analysis was performed using the Mann-Whitney two sample test. Differences were considered to be significant when $P < 0.05$.

Protocol 2: neutralization of endogenous IL-1ra. Rabbits were pre-

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treated with a single i.v. injection of either goat anti-rabbit IL-1ra serum (5.0 mg/ml) (10), or non-immune IgG (5.0 mg/ml) (Sigma Chemical Co., St. Louis, MO), in 1.0 ml saline vehicle or pre-immune serum (10), one week before the induction of colitis. Animals were observed for an additional week before sacrifice. 10 healthy saline-treated control rabbits were housed during the experimental period then sacrificed and assessed similar to the experimentally manipulated animals. Longitudinal sections were obtained and processed as described above. Properties of the goat anti-rabbit IL-ra have been previously described (10); anti-IL-1ra serum neutralized recombinant rabbit IL-1ra bioactivity against IL-1-induced PGE_2 synthesis in primary cultured smooth muscle cells isolated from rabbit colon at an anti-IL-1ra:IL-1ra molar ratio of nearly 1:1 (data not shown). Statistical analysis was performed using an analysis of variance with the Scheffe's multiple comparison procedure. Differences between experimental groups were considered significant when $P < 0.05$.

Histological assessment of inflammation. Representative sections of all colons were analyzed by a single pathologist (C. C. Nast). Acute inflammatory index was calculated as described earlier (21, 22). In brief, morphologic assessment was performed on at least two representative sections from each colon in a blind fashion. The mucosa and submucosa were separately evaluated in a minimum of eight high-power fields (HPFs)[1] each for infiltration by acute inflammatory cells (neutrophils and eosinophils). A semiquantitative score of leukocyte (L)/HPF ranging from 0 to 4+ was used as follows: 0 = 0 or 1; 0.5+ = 2–9; 1+ = 10–20; 1.5+ = 21–30; 2+ = 31–40; 2.5+ = 41–50; 3.0+ = 51–65; 3.5+ = 66–80; 4.0+ = >80 L/HPF. Acute inflammatory index was calculated for each colon by adding the average scores for the mucosal and submucosal evaluations. Chronic inflammation was determined in at least eight high-power fields from each of two representative sections of colon. Mononuclear leukocytes (M) were assessed by a semiquantitative score of 0 to 4+ as follows: 0 = 0–15; 0.5+ = 16–30; 1+ = 31–40; 1.5+ = 41–50; 2+ = 51–60; 2.5+ = 61–70; 3.0+ = 71–80; 3.5+ = 81–90; 4.0+ = >90 M/HPF. Inflammation was restricted to the mucosa; therefore chronic inflammatory index consisted of averaged mucosal scores for each colon.

Rabbit IL-1a and IL-1ra assay. Colonic IL-1α and IL-1ra tissue levels were measured using specific non-cross-reacting radioimmunoassays after tissue extraction as described previously (10, 22).

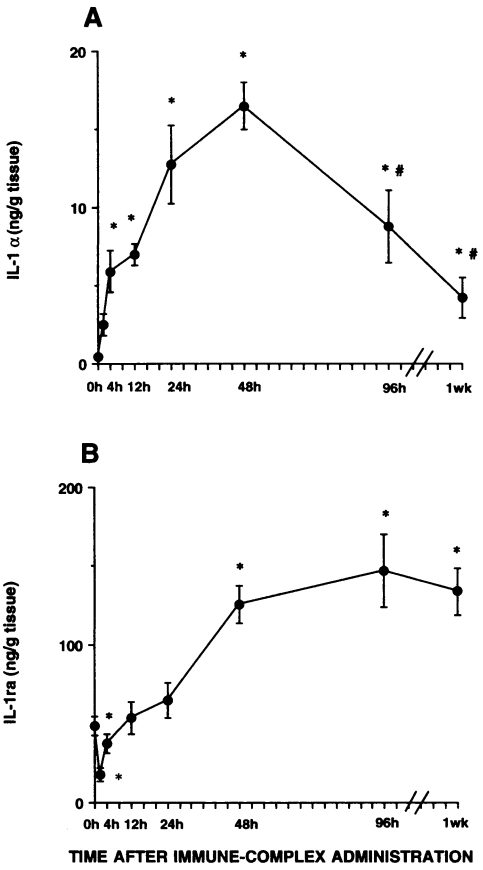

Figure 1. Colonic IL-1 and IL-1ra synthesis during rabbit immune colitis. (*A*) Time course of colonic IL-1α protein levels. (*B*) Time course of colonic IL-1ra protein levels. Colonic IL-1α and IL-1ra tissue levels were measured using specific non-cross-reacting radioimmunoassays as described in text. Data are expressed as the mean±SE of six rabbits. Asterisks indicate statistical differences (*$P < 0.03$ versus 0 h). Statistical analysis was performed using the Mann-Whitney two sample test.

Results and Discussion

Endogenous IL-1ra is known to be produced during a variety of acute inflammatory disorders that appear to be mediated by IL-1 (12). It is speculated that this increase in IL-1ra may represent the host's defense against the proinflammatory effects of IL-1. However, the precise role of endogenously produced IL-1ra, at the present time, is unclear. The purpose of the present study was to investigate the role of endogenous IL-1ra in regulating the biological effects of IL-1 produced in an acute model of intestinal inflammation by answering the following questions: (*a*) when, in relation to IL-1, is IL-1ra produced during an acute inflammatory response? and (*b*) what are the effects of neutralizing endogenous IL-1ra on severity and duration of disease?

To address the first question, we investigated the time course of IL-1ra and IL-1 production in the rabbit model of formalin-immune complex colitis. This model of acute intestinal inflammation is characterized by infiltration of neutrophils and eosinophils with the presence of crypt abscesses, epithelial cell degeneration, mucous depletion, and mucosal necrosis (20, 21). In this model we have previously demonstrated the importance of IL-1 because administration of exogenous IL-1ra markedly suppressed colonic inflammation, edema, and the production of prostaglandins and leukotrienes, associated with intestinal inflammation (22, 23). In the present study, we found that production of IL-1ra in rabbit immune colitis follows a different time-course than that of IL-1. Unlike IL-1, IL-1ra was detected in normal rabbit colonic tissue before the induction of colitis (Fig. 1). IL-1ra levels were significantly decreased immediately after immune complex administration, and did not rise until 48 h after colitis induction, at which time a threefold increase in IL-1ra levels was observed. Elevated levels of endogenous IL-1ra were sustained up to one week following the induction of colitis (Fig. 1 *B*). The mechanism(s) underlying the decrease in IL-1ra levels during the first 2 h after colitis induction are unknown; however, we can speculate that the initial damage to colonic epithelial cells after colitis induction results in a subsequent release of icIL-1ra. The rise in IL-1ra at 48 h preceded a significant decrease in IL-1 production (Fig. 1 *A*) and the resolution phase of colonic inflammation, assessed by measuring leukocyte inflammatory index and my-

1. *Abbreviations used in this paper:* HPF, high-power field; L, leukocyte.

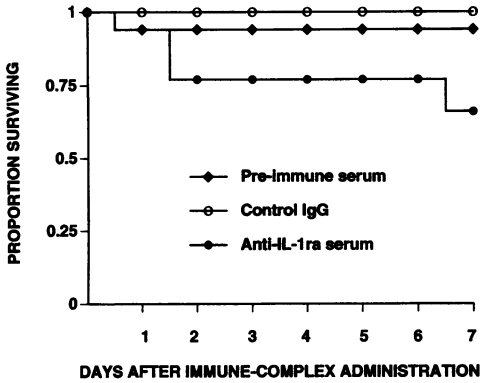

Figure 2. Effects of anti–IL-1ra treatment on mortality in rabbit formalin–immune complex colitis. Anti–rabbit IL-1ra serum (5.0 mg/ml), pre-immune serum, or non-immune IgG (5 mg/ml) were dissolved in 1.0 ml saline. Antisera were administered i.v. one week prior to the induction of colitis and rabbits observed for an additional week. Results are reported as the proportion of surviving colitis rabbits in pre-immune serum (n = 16), control IgG (n = 10), and anti–IL-1ra serum (n = 18) groups with time after the induction of colitis. Mortality was significantly increased in rabbits treated with anti–IL-1ra serum ($P < 0.02$) when compared to pre-immune serum or control IgG-treated animals.

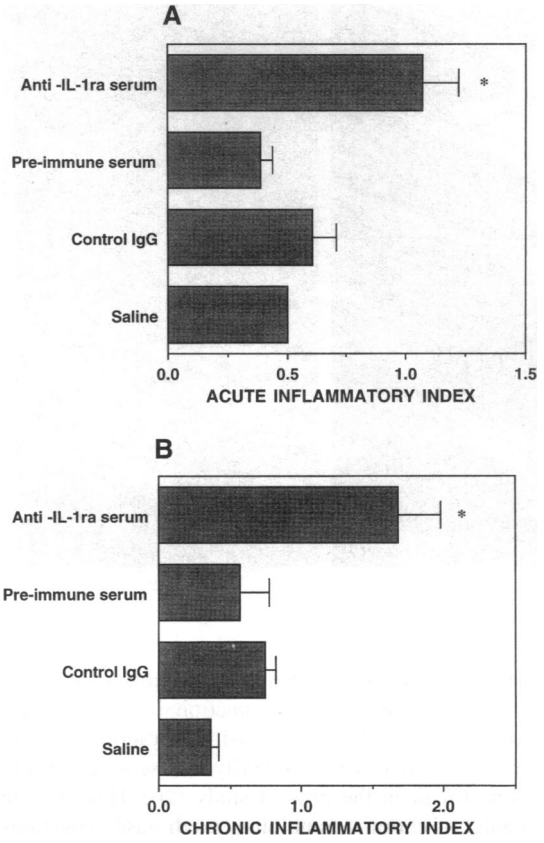

Figure 3. Prolongation of intestinal inflammation after anti–IL-1ra treatment. Infiltration of acute (*A*) and chronic (*B*) inflammatory cells. Rabbits were treated as described in Fig. 2. Histological analysis was performed one week following colitis induction in surviving rabbits in pre-immune serum- (n = 15), control IgG- (n = 10), and anti–IL-1ra serum- (n = 12) treated animals, as well as in healthy saline-treated rabbits (n = 10). Asterisks indicate statistical differences (*$P < 0.03$ versus pre-immune serum or control IgG). Data are expressed as the mean±SE. Statistical analysis was performed using an analysis of variance with the Scheffe's multiple comparison procedure.

eloperoxidase activity (22, 23). It has recently been shown in peripheral blood mononuclear cells that IL-1 mRNA levels peak 3–4 h after stimulation and decrease by 8 h. In comparison, IL-1ra mRNA levels peak 12 h after stimulation, and are sustained for 24–48 h (24, 25). The time course of IL-1 and IL-1ra in the aforementioned in vitro studies is consistent with the in vivo production of IL-1 and its antagonist in the present model of colitis. Taken together, these data suggest that IL-1ra may contribute to homeostatic mechanisms targeted at downregulating and limiting the deleterious effects of IL-1 produced during an acute inflammatory response.

To address the second question, we attempted to define the role of endogenous IL-1ra in the rabbit colitis model using a specific neutralizing anti-serum against rabbit IL-1ra (10). Intravenous administration of anti–rabbit IL-1ra serum significantly increased mortality ($P < 0.02$) when compared with control animals treated with pre-immune serum or non-immune IgG (Fig. 2). 6 of 18 (33.3%) rabbits succumbed to death in the anti–IL-1ra group: three presented with megacolon and perforation, and three with severe colitis and massive necrosis of the distal colon. No macroscopic or histological abnormalities were observed in other organs examined. In a separate experiment, three healthy rabbits were injected with a single dose of anti–IL-1ra serum (5.0 mg/ml). High antibody titers, assessed by immunoprecipitation, were measured in serum samples one week after anti–IL-1ra injection (data not shown). These animals were observed up to 4 wk after anti–IL-1ra administration and showed no evidence of disease. In surviving rabbits, anti–IL-1ra serum significantly increased the number of both acute and chronic inflammatory cells infiltrating the colon, assessed 1 wk after the induction of colitis (Figs. 3 and 4). Furthermore, elevated colonic IL-1 levels were detected in anti–IL-1ra serum-treated animals when compared with controls (Table I). These data suggest that blockade of endogenous IL-1ra may augment and prolong the effects of IL-1 produced during acute intestinal inflammation. It is tempting to speculate that IL-1ra may have an important role in the pathogenesis of chronic inflammatory diseases in humans as well as in the mortality associated with acute inflammatory diseases.

Although we can not exclude the possible contribution of endogenously generated IL-1ra anti–IL-1ra immune complexes to the exacerbation of colitis, this event is unlikely for the following reasons. (*a*) Generation of intestinal inflammation in the present model is dependent on immune complexes in antigen excess (20, 21); given the amount of anti–IL-1ra serum administered to the animals (5.0 mg IgG) and peak levels of IL-1ra measured following induction of colitis (~ 150 ng/g tissue), endogenous immune complexes would be generated in antibody excess. (*b*) Administering only formalin enemas to animals pretreated with anti–IL-1ra serum did not generate characteristic colonic inflammation, indicating that endogenous immune complexes do not produce colitis (data not shown). (*c*) Increasing the dose of exogenous immune complexes does not augment the infiltration of chronic inflammatory cells into colonic mu-

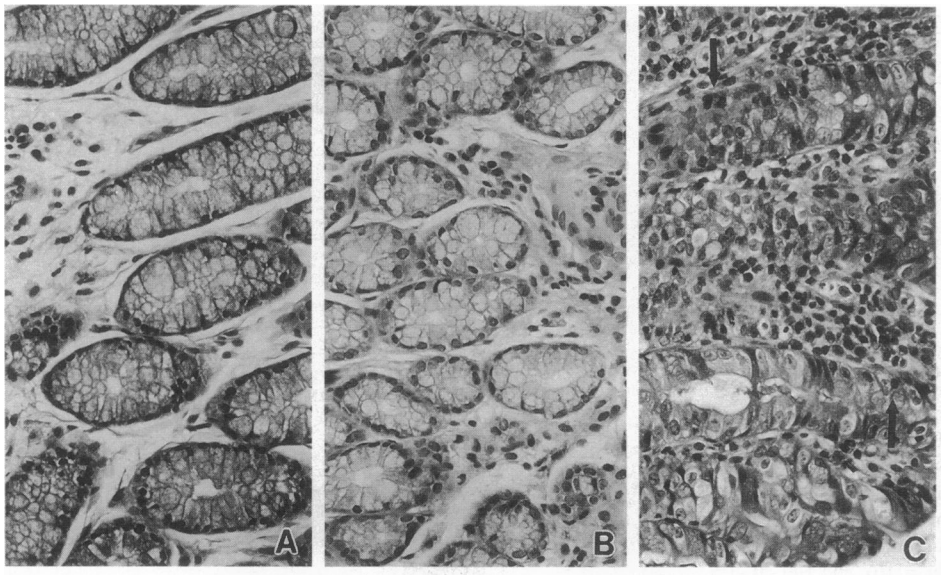

Figure 4. Representative sections of colonic mucosa from rabbits in different treatment groups. (*A*) Pre-immune serum. There are few acute and chronic inflammatory cells within the lamina propria. The glands have normal epithelial cells. (*B*) Control IgG. There is a slight increase in mononuclear leukocytes with few acute inflammatory cells. (*C*) Anti–IL-1ra serum. There are significantly more chronic, and scattered acute, inflammatory cells. Glands are focally infiltrated by mononuclear leukocytes (*arrows*) and show partial mucous depletion. Hematoxylin and eosin ×175.

cosa (F. Cominelli, unpublished data). In addition, in a mouse model of chronic granulomatous inflammation, in which disease severity may be mediated by IL-1, administration of anti–mouse IL-1ra serum increases the severity of disease consistent with the results found in the present study (26). However, in the mouse model of chronic granulomatous disease exogenous IL-1ra administration is ineffective in reducing the severity of the inflammatory response (27).

Endogenous IL-1ra may not be the only factor which regulates the actions of IL-1 produced in disease. Recent in vitro studies have shown that IL-1 receptor type II may function as a decoy target for IL-1 by inactivating IL-1 upon binding, and preventing IL-1 induced signal transduction through the IL-1 receptor type I (28). Thus, the presence of multiple pathways for regulating IL-1 activity during disease states indicates the physiologic importance of balancing this system. In conclusion, the present study provides strong evidence for a pathophysiological role of endogenous IL-1ra in controlling the actions of IL-1 during an acute inflammatory response. Furthermore, our data raise the possibility that defective production of endogenous IL-1ra may be of pathogenic importance in human lethal and chronic inflammatory diseases.

Table I. Colonic IL-1 Levels After Anti–IL-1ra Treatment

	Saline	Control IgG	Pre-immune serum	Anti–IL-1ra serum
IL-1α (ng/g colon)	< 0.4	2.8±0.8	4.5±1.7	11.1±3.7*‡

Rabbit IL-1α was measured in colonic tissue homogenates using a specific radioimmunoassay as in Fig. 2. IL-1α levels were significantly increased in rabbits treated with anti–IL-1ra serum compared with control IgG (*$P < 0.03$) or pre-immune serum (‡$P < 0.05$). Data are expressed as the mean±SE. Statistical differences were determined as described in Fig. 3.

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